

NON-FOOD CHARACTERIZATION/PROCESS/PRODUCT RESEARCH

Panel Manager - Dr. James Linden, Colorado State University

Program Director - Mr. Jeffery L. Conrad

Agricultural commodities can provide the raw materials for production of numerous industrial and consumer products such as lubricants, fuels, paints, detergents, biodegradable polymers, textile fibers, fiber composites, pharmaceuticals, and various other commodity and specialty chemicals. The Non-Food Characterization/Process/Product Research program supports research on improved methods for producing existing non-food, agriculturally-derived products and on developing new, non-food uses for agricultural commodities. Research seeks to better understand properties of agricultural materials related to their quality, value, and processing characteristics and to develop innovative products and processes for conversion of agricultural materials to non-food products.

This program also supports biofuels research directed toward understanding and overcoming factors which limit the technical and economic efficiency of production of alcohol fuels and biodiesel. Supported research focuses on the physiological, microbiological, biochemical, and genetic processes and mechanisms controlling the biological conversion of agriculturally important biomass material to alcohol fuels.

2001-00669 Acquisition of an Automated Crystallization Workstation

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Equipment Grant; Grant 2001-35501-10689; \$49,000; 1 Year

The atomic structure of a protein can be used to decipher functional information necessary to understand how proteins interact with ligands to facilitate complex cellular events. The same high-resolution structural models also make it possible to rationally design proteins with specific substrate binding and enzymatic activities for biotechnology applications. We are using X-RAY crystallography to generate atomic structural models for a wide range of proteins. These proteins include (1) cellulases that will be used to increase biofuel production from crop plant residues, (2) antibody medications manufactured from a novel tobacco-based expression system, and (3) a signal recognition particle (SRP) that directs newly synthesized proteins to developing photosynthetic membranes in plants. The results of these structure-based studies will be used to improve and expand the use of crop plants for biofuel production and for the manufacture of protein-based medications. In addition, our studies of chloroplast S.P. mediated protein targeting will likely provide the fundamental information required for chloroplast bioengineering. In order to keep pace with the large number of isolated proteins and complexes described in this proposal, we request a crystallization workstation that will provide the automation necessary to quickly establish optimal conditions for protein crystal growth. In this context, Sakon's laboratory represents the only site for conducting crystallographic studies in the state of Arkansas. The automated workstation is necessary to position the University and the State for the post-genomics era, a time in which

biotechnological applications of genomic data will bring about an increased demand for high-resolution structural information.

2001-01450 Saccharide-Fatty Acid Esters: Value Added Products Derived Via Biocatalysis

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Standard Strengthening Award; Grant 2001-35504-10756; \$92,665; 2 Years

The overall goal is to improve the rate of production of a "green" process to form saccharide- (sugar-) fatty acid mono-esters from inexpensive agricultural feedstock: fatty acids and sugars. The process involves enzymes (lipases), low temperatures and pressures, and is solventless. The products, important biodegradable emulsifiers used in the foods and cosmetics industries, are "value-added" agricultural commodities. Previously, the investigator demonstrated that the main deterrent to a rapid reaction rate, the poor co-solubilization of sugar and fatty acid reactants, was overcome when significant amounts of the product were present. The investigator "engineered" the reaction medium composition and reaction conditions to take advantage of this result, yielding the highest production rate yet reported. Because a saccharide/ fatty acid mole ratio of 1:2 was employed, further downstream separations were required to isolate the product. The goal of this proposal is to achieve 100% conversion of (both) reactants using a similar operational approach as before, with a 1:1 reactant mole ratio, to eliminate the need for further purification. In addition, the role of water, a reaction product that reduces the product conversion, in the kinetics, and its distribution between the medium's sub-phases, will be determined. Furthermore, a kinetic model, based on the well-known Ping Pong Bi Bi model, will be developed. Both the model and the other proposed experiments will help identify reaction conditions to optimize the batchwise production of ester and design and operate a continuous bioreactor, the long-term goal of this project.

2001-01557 Organometallic Carbohydrate Chemistry: New Technology for the Use of Agricultural Feedstock

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Standard Strengthening Award; Grant 2001-35504-10735; \$133,000; 2 Years

A program is described to develop transition metal mediated transformations of carbohydrates as a general method that could be used for efficient production of useful chemical products. Agriculture excels at the production of carbohydrates through growing of annual domestic crops and forest products, yet only about 2% of the U. S. chemical feedstock supply comes from agricultural sources. The primary reason for this limited use of carbohydrates as chemical building blocks is not economic, but technological. Few simple, selective, high yield methods for the modification of the native carbohydrate structures exist. The use of transition metal complexes to promote selective structural modification of simple carbohydrates is a method that we believe will bridge this technology gap. The main objective of this program is to develop new, transition metal mediated transformations of agricultural carbohydrates. The expected

results of this program will clearly be applicable to the manufacture of useful carbohydrate based products such as detergents, surfactant, and polymers.

2001-01410 Engineering Ethanol for Fuel Ethanol Production

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Grant 2001-35504-10669; \$300,000; 3 Years

In the U.S., corn starch contributes more almost 2 billion gallons of ethanol each year, over 1% of the total automotive fuel. With the toxicity problems of MTBE, ethanol may soon be needed in much larger volumes as an oxygenate, octane enhancer and fuel extender. Although grain-derived ethanol can expand, competing markets for food would prevent expansion to the levels required to replace imported petroleum for fuel. Over half of the petroleum used in the U.S. is imported, an amount equivalent to that burned as automotive fuel. The monetary cost of imported petroleum exceeds the trade imbalance for any single nation while the societal cost of our strategic dependence includes the subordination of human rights issues and the domination of military/foreign policy. The production of inexpensive sugar from lignocellulosic agricultural residues is the key to replacing imported petroleum. Sugars derived from renewable plant residues can serve as alternative feedstock to produce fuel ethanol, bulk chemicals and biodegradable plastics. Costs of these biological processes are currently a major barrier. The proposed research focuses on the development of genetically engineered bacteria that produce a portion of the enzymes for the solubilization of cellulose, arguably the most expensive step. The use of these strains will decrease the cost of cellulose hydrolysis to soluble sugars for ethanol production and other uses such as lactic acid and propanediol for plastics, calcium-magnesium acetate (CMA) as a replacement for road salt, and other chemicals.

2001-01429 Process Technology for Scaleup of Enzymatic Catalysis in Organic Media

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Grant 2001-35504-10686; \$183,000, 2 Years

The use of enzymes as catalysts for organic phase reactions offers the potential for the transformation of agricultural feedstock into high value-added products. Unfortunately, in organic media, the activity and stability of enzymes has been shown to be a strong function of the amount of water present. Precise control of the water content is required to fully realize the potential of these systems. The objectives of this research are to: (1) Develop an in-situ sensor that has the capacity to measure the water content of mixed organic solutions (as would be found in an enzymatic reactor) within a minute of process changes. (2) Develop commercially viable polymeric membranes capable of selectively removing water from an organic-phase enzymatically-catalyzed reaction mixture. (3) Integrate these two technologies to demonstrate the ability to have rapid sensing and control of water content.

The results achieved from this research have direct impact on enzymatic reactions in organic media. This work will result in improved enzyme stability and increased reaction rates. Ultimately, it may provide a means to the full-scale commercialization of

technology for the transformation of agricultural feedstocks into biolubricants, flavors, fragrances, pharmaceutical intermediates, and other value-added compounds.

2001-01180 Acquisition of a Scanning Fluorescence Microplate Reader to Enhance Research in Agricultural Biotechnology

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Equipment Grant; Grant 2001-35501-10717; \$17,375; 1 Year

This is an equipment grant for purchasing a scanning fluorescence microplate reader for use by a group of researchers at the University of Hawaii who are engaged in agricultural biotechnology research. These researchers all have funded research projects that require the use of the fluorescence microplate reader. The projects include (1) Bioengineering studies of recombinant protein production from transgenic plant cells, (2) Genetic engineering approach to aid recovery of recombinant proteins from transgenic plants, (3) Development of novel fluorescent biosensors by protein engineering to assure safety of food supplies, (4) Bioreactor and metabolic engineering of microalgae for enhanced production of high-value nutraceuticals, (5) Production of monoclonal antibodies against myostatin and investigation of the mechanism of action of myostatin, (6) The role of mimosine in bacteria-plant interactions in the *Leucaena* rhizosphere, and (7) Molecular analysis of Rhizobium-Tree Legume Symbiosis. These projects address important problems in emerging areas of agricultural biotechnology that have a great impact on value-added processing, animal growth, and sustainable agriculture and agroforestry. Currently, there is no other similar instrument available at the University of Hawaii. The availability of the plate reader will certainly strengthen the research capability of the participating agricultural biotech researchers at the University of Hawaii and help them remain competitive in their respective fields in the future. The acquisition of the instrument will also broaden the scope of their research and allow them to move into new and exciting research areas.

2001-01469 Characterization of *Clostridium beijerinckii* BA101 Hyper-Butanol Producing Mutant

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Grant 2001-35504-10668; \$221,300; 3 Years

Recent economic studies funded by the Illinois Corn Marketing Board have indicated that the use of the *Clostridium beijerinckii* BA101 hyper-solvent producing strain in combination with pervaporative solvent recovery plus glucose or starch as the substrate is an approach which is competitive to the petrochemical route for producing the solvents, acetone and butanol. However, since the *C. beijerinckii* BA101 strain was produced using chemical mutagenesis, the molecular basis for why this strain produces enhanced levels of acetone and butanol is not fully understood. Several key enzymes in the solvent forming pathway of *C. beijerinckii* BA101 have been shown to have elevated levels of activity and expression, but these findings do not fully explain the metabolic activities of *C. beijerinckii* BA101. The objectives outlined in this proposal include an

examination of glucose transport in *C. beijerinckii* BA101 and NCIMB 8052, specifically the functions and involvement of sugar transport in global regulation of solvent production, the genetic manipulation of *C. beijerinckii* BA101 and NCIMB 8052 for increased butanol production, and fermentative and physiological characterization of the newly constructed *C. beijerinckii* BA101 derivative strains. These experiments are expected to increase our understanding of and the potential for solvent production by *C. beijerinckii* utilizing corn and corn processing wastes.

2001-01448 Solid-Liquid Equilibrium of Fats & Oils-Pursuant Phase Change Material Applications

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Grant 2001-35504-11208; \$140,000; 2 Years

Alternative uses of fats and oils are becoming more and more important as the growth in fat and oil supplies rapidly exceed demands for these products in traditional markets. Phase change material (PCM) applications are most promising for two reasons: (1) fats and oils have natural melting points at useful PCM application temperatures and (2) large PCM demands could potentially consume essentially all excess fats and oils at premium prices >\$0.30 per pound. Successful applications of fats and oils as PCM chemicals could have positive ramifications toward essentially all commodity grain crops since the market for PCM chemicals is large enough to promote increased use of farmland for oil crop production.

Fundamental and applied research of this USDA-funded project will address the most critical aspect of competitive applications as PCM chemicals-the fundamental understanding of the melting properties of fats and oils to allow transesterification processes to convert >95% of natural fats and oils into high-performance PCM chemicals. To date, successful applications of fat and oil derivatives as PCM chemicals have been based on highly refined derivatives where only 20%-40% of the natural fat/oil is converted to the PCM chemical.

Ultimately, the impact of this research extends beyond the development of new fat and oil markets. Thermal storage by PCM chemicals allows energy to be conserved leading to reduced air conditioning and heating costs for consumers, reduced generation of greenhouse gases, and reduced demand for electrical power including solutions to the energy crisis experienced by the West coast.

2001-01464 Butanol Tolerance in Clostridia

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Grant 2001-35504-10670; \$194,000; 3 Years

Our long-range objective is to improve solvent producing clostridia so that they may again be used in cost-effective fermentation for solvent and biofuel production from renewable agricultural and industrial sources. To reach this goal we need to understand, and thus limit, the toxic effects of the fermentation end product, butanol. We will employ the solvent producing strain, *Clostridium beijerinckii*, and a mutant strain whose butanol

tolerance is associated with decreased ability to detoxify the toxic metabolic intermediate, methylglyoxal, and with increased methylglyoxal levels in the culture. Our goal is to establish the molecular mechanism(s) by which these toxic metabolites affect the growth and metabolism of solvent-producing clostridia. We will test the working hypothesis that protein modification by methylglyoxal (glycation) is related to butanol susceptibility of the bacteria. We will assess the functional integrity of the mutant and wild type cell membranes, by measuring their ability to maintain transmembrane ion gradients when challenged with butanol. A second group of proteins that appear to be sensitive targets of butanol are cell wall-associated enzymes important for normal separation of cells during growth. We will investigate the effects of butanol and degree of protein glycation by methylglyoxal on the activities of these enzymes. We will also seek butanol tolerant mutants of *Clostridium acetobutylicum* ATCC 824, whose genome has been sequenced, and investigate promising mutants by the methods developed with *C. beijerinckii* strains.

2001-01417 Mechanisms for the Adsorptive Separation of Chemicals from Renewable Resources

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Standard Strengthening Award; Grant 2001-35504-10667; \$154,300 3 Years

At the same time that pressures are mounting to reduce the need for petroleum-derived chemicals, there is a growing understanding of the functional properties of biologically derived chemicals. Thus, renewable resources are poised to emerge as a significant source of chemicals for medicine, agriculture, food, and industrial applications. To realize this potential however, inexpensive methods must be found to overcome the "separations bottleneck." Separation methods cannot be directly transferred from petro-chemical processing because distillation, the predominant method for separating petro-chemicals, is impractical for less-volatile bio-chemicals. Separation methods emerging from studies of molecular recognition (e.g. polymer imprinting and supramolecular chemistry) offer exciting opportunities, but these methods tend to be excessively expensive for bulk chemical manufacture. We propose to apply the principles and tools of molecular recognition to learn how subtle differences in binding interactions can be exploited for cost-effective adsorptive separations. Our focus is on oxygenated aromatic compounds (OACs) since these compounds offer a variety of functional properties and can be obtained from abundant natural resources.

We propose fundamental studies to characterize adsorptive interaction mechanisms. Specifically, we propose to use small molecule analogs of the putative adsorption sites and to probe solute analog binding mechanisms using various spectroscopic techniques. Further, we propose to study the thermodynamics of solute adsorption onto various sorbents and to develop correlations between solute:sorbent and solute:analog binding. Finally, we propose to examine various approaches to separate OACs by exploiting subtle differences in acid-base interactions.

2001-01463 Bio-Composites From Engineered Bio-Fibers and Bio-Plastics

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Grant 2001-35504-10734; \$232,500; 3 Years

New environmental regulations, societal concerns, and growing environmental awareness have triggered the search for new products and processes that are compatible with the environment. Bio-Composite Materials made from natural/bio-fibers and bio-plastics can supplement and eventually replace petroleum based composite materials for structural applications offering new agricultural, environmental, manufacturing and consumer benefits. This proposal seeks to derive sustainable eco-friendly bio-composite materials from engineered natural fiber (ENF) and developed soy protein based bio-plastic through a novel processing approach. Kenaf and Pineapple leaf fibers will be subjected to low-cost ammonia fiber explosion (AFEX) and/or alkali treatments (Engineering of Bio-fibers) to improve the bio-fiber adhesion to bio-plastic matrix. The soy-based bio-plastics will be developed through reactive extrusion processing. This novel processing approach is named Bio-Composite Stampable Sheet (BCSS) Processing and consists of: (1) the intermingling of various combinations of chopped "engineered natural fibers" with powdered soy-based bio-plastic; (2) application of an electrical field to align the bio-fibers; (3) partial consolidation via sintering the bio-plastic powder to bridge and hold the fibers in place; and (4) finally producing bio-fiber reinforced bio-plastic composite in either thin or thick sheets suitable for further compression molding. The materials and process elements of this project will be synergistically investigated to achieve a bio-composite which is at least equal in performance and lower in cost to glass reinforced composite materials for structural applications. The success of this project will allow for supplementing the use of petrochemical based materials with renewable resource materials with long range benefits to agriculture, the environment, industry and society and will create a process for a new generation of bio-composites in applications such as car body interiors, rigid packaging and interior panels for housing.

2001-00508 Conference Entitled: 2001 International Nonwovens Symposium

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Conference Grant; Grant 2001-35504-10524; \$8,000; 1 Year

The conference was part of the 46th Annual Meeting of the Beltwide Cotton Conferences held in Anaheim, California, January 9-13, 2001. The International Nonwovens Symposium program encompassed 29 oral presentations and 5 poster presentations, totaling 34 papers - authored by over 70 authors and co-authors. They were presented in 8 sessions on January 12 and 13, 2001 at Marriott Anaheim, Anaheim, California. A planning meeting was held on January 11, 2001. Scientists from academia, government and industry participated. All the scientists were hands-on scientists presenting and discussing their latest research findings. The conference was a forum for networking, freely exchanging ideas and information. The National Cotton Council provided the site (meeting rooms, audio-visual equipment, etc.). Dr. D. V. Parikh, Head Nonwovens Research at SRRC, USDA, New Orleans, Louisiana took the lead in inviting distinguished scientists to present their papers at the seminar.

To organize excellent nonwovens symposiums. The nonwovens symposiums held annually are the authentic resource for (a) recent developments in the technology of

nonwovens that sustain future growth of the industry, and (b) dissemination of the latest information.

We organized the Fourth Nonwovens Symposium whereby scientists, researchers, manufacturers and end users came together and discussed the latest developments in the nonwovens industry. They also discussed the ways to increase the use of cotton, waste cotton and other natural agricultural products such as jute, kenaf and flex in the manufacture of nonwoven products.

2001-00862 Applications of Natural Products as Wood Protectants for the Lumber Industry

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Seed Grant; Grant 2001-35501-10682; \$74,961; 2 Years

Timber protection from fungi, termites and oxidation is currently based primarily on the use of highly toxic mineral and petroleum byproduct fungicides and insecticides. Arsenic, creosote, and toxic pesticides are still widely used all over the world, including the United States, as wood preservatives. Analytical studies in several countries have shown serious contamination of soil, ground- and surface water with various toxic components originating from commercial wood preservatives. In addition these highly toxic wood preservatives have been found to directly threaten the health of timber and lumber industry workers as well as nearby populations. Consumers using timber products protected with toxic chemicals can also be affected. There is an urgent need to discover and develop novel, non-toxic materials for timber protection. Natural products and their analogs are promising candidates for such materials.

The primary goal of this project is to develop new, inexpensive and environmentally friendly timber preservatives based on natural product models and their synthetic production from existing agriculture and petroleum streams. Natural products produced by marine plants and invertebrates to protect themselves and which are inexpensive to produce commercially will be examined for their ability to serve as new preservatives for wood products. By increasing the value and life expectancy of low-grade timber products in the United States this project will clearly benefit both the consumer and those industries involved in timber production. The consumer will gain significantly by improved product quality and reduced health hazards from the well-documented risks associated with currently applied, highly toxic wood preservatives.

2001-01462 Investigation of Vegetable Oil-Derived Macromonomers as Copolymers in All-Acrylic Latexes

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Standard Strengthening Award; Grant 2001-35504-10775; \$120,000; 2 Years

Environmental regulations impose restrictions on volatile organic compound (VOC) emissions of architectural coatings as they ultimately contribute to smog formation. The introduction of novel castor oil derived macromonomers into emulsion polymers allows formulation of high performance, low odor, and essentially no VOC

architectural coatings. A new series of vegetable oil macromonomers (VOMMs) are proposed and would be developed by modifying castor oil with locally available fatty acids such as linolenic acid, soybean fatty acids, conjugated linolenic acid (Pamolyn 280), and oleic acids. It is vital that fundamental data be generated regarding monomer reactivity ratios and kinetics of ambient cure crosslinking in order to fully exploit the value of this novel class of VOMMs. This study will provide crucial information regarding the relationship of VOMM properties and structure, and consequently will enhance the contribution of indigenously available vegetable oil fatty acids while reducing the VOC levels of architectural coatings.

2001-01561 Effect of Cellulose Mesophase Structure on Cellulose Fiber Properties

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Grant 2001-35504-10696; \$117,534; 2 Years

This proposal, though quite fundamental in nature, has a number of practical aspects, which will be of benefit to agriculture, industry and the environment. It deals with the modification of a widely used fiber, cellulose. The proposed research, exploiting novel cellulosic systems, as well as advanced analytical techniques, constitutes a unique effort, and the first of its kind to identify and control the physical and mechanical properties of cellulosic fibers. Regenerated cellulose fibers, for example viscous rayon fibers, have much lower strengths than theoretically predicted. This is due in part to the fact that the ordered structures are not well preserved during the fiber forming process. Producing an ordered cellulosic solution that can be readily converted (fiber spinning) into cellulose fibers would significantly eliminate the strength issues currently associated with regenerated cellulose production. The flaws in the forming fibers would be minimized or even eliminated, enabling higher strength cellulose fibers to be produced. In addition, higher spinning concentrations could be utilized permitting reduced operating costs. The present research will develop structures in cellulose fibers that will produce fiber strengths closer to theoretical prediction. A variety of analytical techniques will be employed to study fiber structure and develop methods to alter the structure and approach the desired fiber properties. This will permit adjustments in fiber spinning techniques to obtain the desired fiber performance.

2001-01560 Microcellular Foam Extrusion of Modified Starch Based Composites-Materials, Mechanics and Process Design

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Grant 2001-35504-10681; \$111,600; 2 Years

Supercritical fluid extrusion (SCFX) using supercritical dioxide (SC-CO₂) is an alternative and "green" process technology that is amenable to producing low density rigid microcellular foams from renewable resources such as starch. These foams can subsequently be converted into numerous utility products of different shapes and sizes like cups, plates, trays, loose fills and thermal insulation sheets, otherwise predominantly made from plastics. As compared to unfoamed as well as conventionally foamed materials, the microcellular foams can have high toughness, fatigue life, thermal stability,

reflectability, and low dielectric constant and thermal conductivity. This project proposes to investigate the dynamics of cell nucleation, cell growth and cell stabilization during the continuous SCFX processing of starch based materials into sheets, in which the final cell sizes are less than 50 FL m and cell densities are in the range of 106_1015 cells/cm³. The hydrophobic surface character of the foam sheets will be evaluated after coating them with 100% biodegradable and water repellant formulations containing acylated starch, starch alkenyl succinates or cellulose acetate butyrate (CAB). The thermoforming of the foam sheets into simple tray-like geometrics will also be attempted.

2001-01436 Enhanced Enzymatic Biocatalysis for the Synthesis of Sugar-Containing Biodegradable Copolymers

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New Investigator Award; Grant 2001-35504-10757; \$65,318; 1 Year

Enzymes are protein catalysts that manipulate the construction of various polymers and chemicals in biological world. To learn and use the enzymatic processes occurring in nature for the development of novel biomaterials has therefore been a vigorous research effort for many scientists and engineers. Nevertheless, we are still at the beginning to explore and demonstrate the potentials and merits of enzymes for polymer synthesis. The long-term goal of this research is to develop highly effective enzymatic processes for the production of a new class of sugar-containing polymers that are biodegradable and biocompatible. The products incorporate extremely hydrophilic sugars and relatively hydrophobic lactide, thus afford desirable physicochemical properties in terms of mechanical behaviors, hydrophilicity, and rate of decomposition. These properties are tunable within a wide range by controlling the sugar content; make it possible to satisfy the requirements for a variety of applications including biomedical materials, absorbents, adhesives, cosmetic products, and general disposable polymers.

The proposed work is aimed to promote the production of non-fuel chemicals and materials from agricultural resources. In addition to the raw materials including sugars and lactide, the protein enzymes applied for the polymer synthesis are also produced from agricultural commodities. The use of enzymes will provide simple reactions, clean products, and environmentally friendly processes. The success of this work will ultimately lead to the development of industrial processes and products that are both bio-based, and will considerably enhance the value of agricultural raw materials.

2001-01420 Development of Low-Phytate Soybeans by Phytase Gene Engineering

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Grant 2001-35504-10662; \$180,000; 2 Years

Phytate comprises greater than 60% of stored phosphorus in soybean seeds and is nutritionally unavailable to non-ruminant animals. Undigested phytate is excreted into manure and contributes to environmental phosphorus pollution in areas of intensive animal production. Supplementation of animal diets with phytase, an enzyme that removes the phosphate from phytate, has been shown to improve phosphorus availability in Teed. Supplementation has nor been widely used in the US due to the associated cost.

Reduction of seed phytate levels will provide a cost-effective alternative for improved phosphorus utilization in animal feed. The long-term goal of the proposed research is to reduce unavailable phosphorus levels in soybean seeds to improve their value and utility as feed components. Our strategy is to produce low-phytate soybeans by introduction of a phytase gene. Phytase expression in developing seeds should allow the removal of phosphate groups from phytate prior to harvest. Improved phosphorus availability in soybean will provide improved waste management tools and will enhance agricultural sustainability. The specific project objectives include: (1) analysis of soybean plants containing the phytase gene, (2) measurement of phytase activity and determination of the localization of the phytase enzyme in transgenic soybeans, and (3) genetic evaluation of regenerated plants and determination of phytate levels in seeds.

2001-01418 Quantitative Analysis of Gene Expression During Xylose Fermentation by Yeasts

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Grant 2001-35504-10695; \$200,000 2 Years

Gene expression is the first step in the process that converts genomic messages into enzymes and cellular structures. Expression comprises the conversion of DNA sequences into mRNA. Expression of the 6000 genes found in yeast is closely synchronized with cellular metabolism. As cells grow and adapt to their environment, different sets or levels of these proteins are formed as needed to assure survival. For example, as the carbon source changes, cells must induce different metabolic pathways for growth. The yeast *Saccharomyces cerevisiae* is well adapted to use of glucose and other six carbon sugars to make ethanol, but it does not metabolize xylose and other five carbon sugars. This is unfortunate because xylose is a major constituent of agricultural residues. If *S. cerevisiae* could ferment xylose efficiently to ethanol, corn stover, wheat straw, soybean residues and wood waste could become sources for renewable transportation fuels. This would give farmers a new source of income, and the resulting fermentation industry would provide jobs in rural areas. It is possible to use molecular biology to engineer the expression genes from xylose fermenting yeasts such as *Pichia stipitis* into *Saccharomyces*. Research has revealed several of the proteins that are needed for xylose metabolism, but essential steps are still unknown. This research will use tools to quantitatively measure gene expression on a genome-wide scale. By studying the genome-wide expression of *S. cerevisiae* and *P. stipitis* genes of wild-type and genetically engineered cells growing on glucose and xylose we will identify the remaining genes.